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The composition of the BLOOD, URINE and SALIVA of DAIRY COWS as affected by extremes in the level of protein feeding

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FOREWORD

This phase of a prolonged Protein Feeding Experiment, other parts of which have previously been published, was suggested to the writer by Dr. J. F. Lyman, Professor of Agricultural Chemistry now retired, of The Ohio State University: to whom the writer is also indebted for valuable advice. I am likewise indebted to Dr. Russell Conrad, present staff member of the Dairy Science Department, who encouraged and arranged for its publication.

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THE COMPOSITION OF THE BLOOD, URINE AND SALIVA OF DAIRY COWS AS AFFECTED BY EXTREMES IN THE LEVEL OF PROTEIN FEEDING

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INTRODUCTION

In connection with investigations previously reported (47) in which one group of dairy cows was fed for long periods on a ration of nutritive ratio 1:13, extremely low in protein, while another group was fed on a ration of opposite extreme with a nutritive ratio of 1:2, analyses were conducted to show the effect of such feeding on certain body fluids: blood, urine and saliva.

For details regarding the feeding of these animals and the effect of such feeding on the animals and the amount and composition of the milk produced the reader is referred to the other publications in this series (43) (47).

It seemed reasonable to assume that these fluids which play so important a part in the distribution and elimination of the products of digestion and metabolism would show important changes in composition as the result of such drastic changes in feeding as have been practiced in this experiment.

Since protein is essentially nitrogenous in nature much of the research effort was directed toward the determination of the various nitrogenous constituents of these fluids though other determinations which might be relevant were also made. The analyses of the saliva were less detailed.

COMPOSITION OF BLOOD: Procedures

The samples of blood for analysis were obtained from the jugular vein. About 100 ml. were usually drawn for a sample into a clean dry glass stoppered bottle containing 0.25 gram of finely divided potassium oxalate as an anti-coagulant. The bottle was continuously shaken as

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the sample entered so that coagulation was usually prevented. Additional samples of the blood without the oxalate were also taken for determinations to be conducted on the blood serum. The blood samples were usually taken in the forenoon 3-5 hours after the cows had eaten their morning feed allowance and had access to water. In the cow much of the food is retained for a considerable time in the rumen. Digestion goes on slowly and may be considered as a continuous process rather than intermittent as with simple stomached animals. For this reason the time of sampling the blood would probably have less influence on the observed composition than has been reported in the case of work done on dogs and humans.

A protein free filtrate of the blood was prepared at once in quantity, according to Haden's modification (27) of the Folin and Wu method (22). This was preserved by the addition of toluene, about ½ ml. per liter and refrigerated.

The total non-protein nitrogen was determined by digesting 10 ml. portions of the protein free filtrate representing 1 ml. of blood with a diluted mixture of sulphuric and phosphoric acids, containing a trace of copper. A few drops of 30 percent hydrogen peroxide were added in final stages of digestion as recommended by Koch and McMeekin (32). After all organic matter was apparently destroyed and the sample clear, heating was continued for at least 2 minutes. After cooling, the sample was diluted to about 20 ml. and made alkaline with 30 percent sodium carbonate solution. The ammonia was aspirated into a measured quantity of N/140 sulphuric acid by the Van Slyke and Cullen (54) procedure. The excess of acid was titrated with N/140 ammonia, using as indicator a very dilute and carefully neutralized solution of methyl red.

Urea was determined on 10 ml. portions of the protein-free filtrate. The urea was decomposed by means of the enzyme, urease, and the resulting ammonia aspirated and titrated by the same procedure described in the case of the total non-protein nitrogen. Use has been made both of commercial urease tablets and of an extract of the Jack Bean in 25 percent alcohol as originally suggested by Marshall (35) and improved by Van Slyke and Cullen (53) (54) as sources of urease.

Uric acid was separated from the protein-free blood filtrate by precipitation with silver lactate and subsequent treatment of the precipitate with a 10 percent solution of sodium chloride in N/10 hydrochloric acid as recommended by Folin et al. (23). The determination was then completed by the Benedict colorimetric procedure.

Creatine in 5 ml. portions of the protein-free filtrate was converted into creatinine by heating in an autoclave with 1 ml. of N hydrochloric acid at 130° C. for 20 minutes.

The total creatine and creatinine was then determined colorimetrically by means of the color developed in an alkaline solution of picric acid in comparison with that developed by a standard creatinine solution under the same conditions substantially by the method of Folin (21).

Two methods have been used for the determination of amino-acid nitrogen. That of Folin and Wu (22) was first used. In the later stages of the work Danielson's modification (13) of the Folin method was adopted.

The amid nitrogen was determined by the method of Bliss (8).

Blood sugar was determined on 10 ml. portions of the blood filtrate by the Bugbee and Simond (9) modification of the Shaffer and Hartman (51) method, 5 ml. of the Shaffer and Hartman micro-reagent were used, and N/140 sodium thiosulphate was used in the titration.

Chlorides were determined on 10 ml. portions of the protein-free blood filtrate by the method of Whitehorn (55).

Inorganic and total acid-soluble phosphates were determined on the whole blood by the method of Fiske and Subbarow (16).

The carbon dioxide capacity of the blood serum was determined by the method of Van Slyke and Cullen (54).

The calcium content of the blood serum was determined by the Clark and Collip (12) modification of the Kramer and Tisdall method (33).

RESULTS AND DISCUSSION OF BLOOD ANALYSES

At least four sets of published results are available which help to give an idea regarding the composition of the blood of dairy cows under normal conditions.

Robinson and Huffman (48) reported the range of values observed for the mineral composition of more than 100 samples of the blood of normal, mature cattle. Hayden and Fish (29) also gave the range and average values of a large number of samples of normal cow's blood, and include most of the other generally useful determinations as well as the minerals.

Allardyce, Fleming, Fowler and Clark (1) presented considerable data regarding the normal values for various ingredients of cattle blood. They also presented a table showing that for most of the constituents the

normal values for cattle blood agree quite closely with the generally recognized normal values for these same constituents occurring in human blood.

Anderson, Gayley and Pratt (2) have also reported studies on bovine blood. Dairy animals of all ages and both sexes are included in their report. Most of the samples however came from animals less than one year old. Six samples only were taken from mature cows. Their analyses included most of the determinations on blood which we have carried out, and their results agree well with the other values quoted.

TABLE 1.—Nitrogenous Constituents in Blood.
(Milligrams per 100 cc. Blood)

Ingredients	High Protein (1:2 Nutritive Ratio)				Low Protein (1:13 Nutritive Ratio)			
	No. Dets.	High-est	Low-est	Av.	No. Dets.	High-est	Low-est	Av.
Total non protein nitrogen	10	64 4	36.8	44.0	8	30.0	11.6	20.0
Urea nitrogen	10	56 5	18.0	27.5	8	3.5	0.0	2.5
Uric acid nitrogen	12	2 4	1.9	2.0	9	2.7	2.0	2.4
Total creatinine nitrogen	10	5.3	4.0	4.7	9	5.4	2.1	4.2
Amid nitrogen (Bliss Method)	12	135.0	103.0	120.0	8	123.0	100.0	109.0
Amino acid nitrogen	7	6.2	4.5	5.1	8	6.6	4.7	5.3

TABLE 2.—Certain Non-nitrogenous Constituents in Blood.
(Milligrams per 100 cc. Blood)

Ingredients	High Protein (1:2 Nutritive Ratio)				Low Protein (1:13 Nutritive Ratio)			
	No. Dets.	High-est	Low-est	Av.	No. Dets.	High-est	Low-est	Av.
CO_2 absorbing power (Blood Serum)	6	62.0	50.0	57 0	6	75.0	53.0	61.0
Blood sugar	10	78.0	44.0	60.2	7	79.0	43.0	63.5
Calcium (Blood Serum)	7	12.6	9.0	10.9	7	12.3	10.0	11.0
Acid soluble phosphorus	10	15.3	8.4	11.4	7	13.0	8.2	9.9
Inorganic phosphorus	10	7.4	5.0	6.2	7	6.5	4.5	5.4
Chlorine	12	362.0	255.0	293.0	10	395.0	264.0	314.0

The data from our experiments are presented in Tables 1 and 2 concerned respectively with nitrogenous and non-nitrogenous constituents of the blood.

The total non-protein nitrogen for the high protein group is seen to be more than twice as great as the corresponding value for the low protein fed animals.

The value for urea nitrogen shown in the second line is seen to be about 11 times as great for the high as for the low protein group. The urea which is included in the non-protein nitrogen is probably responsible for most of the difference noted there, since none of the other nitrogenous constituents show any striking difference between the two groups. The uric acid nitrogen averages slightly higher for the low protein group reminding one of a similar result in the case of the milk from these cows (43) and a somewhat higher excretion in the urine.

Little difference is to be noted in the values for the non-nitrogenous constituents as reported in Table 2 except that the somewhat higher value for chlorine in the low protein group may have a definite connection with the somewhat greater urinary chlorine excretion of the low protein cows. Other than total non-protein nitrogen and urea the observed values come well within the limits of the normal values found by the other workers cited and also agree quite well with corresponding values in human blood as shown in a table published in the Hawk and Bergeim text (28).

COMPOSITION OF URINE: Procedures

For the collection of urine samples the procedure described by Nibler and Turner (42) was followed. A collection at about 3 hour intervals over a 24 hour period was first conducted. The results appearing in Table 3 show an approximately uniform rate of urine production. Total nitrogen determinations conducted on these samples likewise showed a uniform distribution of voided nitrogen. For later work the urine was collected four times in succession at two hour intervals the first collection being discarded and the other three from each cow combined to serve as the samples representing ¼ of a 24 hour period. The sample was thoroughly mixed and measured. An ample portion was preserved with toluene at the rate of about 1 ml. per liter and stored in the cooler near the freezing point. Such determinations as were noticeably affected on standing, particularly the ammonia nitrogen, were run at once on the fresh untreated samples.

TABLE 3.—Volume of Urine Collected at Approximately Three Hour Intervals

Sample No.	Collection starting at:	Low Protein		High Protein		
		Cow 293	Cow 301	Cow 292	Cow 329	Cow 332
		(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
1	1 P. M.	1600	1250	2320	1540	2330
2	4 P. M.	760	340	1040	1070	----
3	6 P. M.	520	560	1560	600	1000
4	9 P. M.	680	580	1480	1360	2440
5	12 Midnight	620	1220	2100	960	1900
6	2:30 A. M.	620	480	1030	1260	1460
7	5 A. M.	670	580	1860	920	1360
8	8 A. M.	500	1320	1880	620	1540
9	11 A. M.	440	1040	1040	1160	960
10	1:30 P. M.	860	2380	1880	1090	1750
Totals*		5670	8500	13870	8980	12410

*Totals—Samples 2-10 inclusive 24 hr. period.

Since proteins consist largely of nitrogen but also contain sulphur and phosphorus special attention was given to these elements in our analyses though others were included. Many of the methods used are similar to those used for the same ingredients in blood.

Total nitrogen was determined on 5 ml. portions of filtered urine by a macro-Kjeldahl procedure.

Ammonia was determined by the aeration of 25 ml. portions of fresh urine made alkaline with 5ml. of 20 percent solution of sodium carbonate into a measured excess of N/140 sulphuric acid and back titration of the remaining acid with N/140 ammonia. Three drops of caprylic alcohol were added to each sample to prevent foaming. Ammonia determinations must be carried out at once on the fresh samples as greatly increased values are likely on samples which have stood for only a few hours regardless of preservation or refrigeration. The indicator used in the titration was a dilute and carefully neutralized solution of methyl red.

Urea was determined on samples of urine ranging in size from 1/10 ml. to 5 ml. depending on the urea content of the sample as judged by previous work or a preliminary determination. The sample was diluted

to 15 ml. then treated with 5 ml. of 10 percent Jack Bean extract in 25 percent alcohol, incubated in a water bath at about 40-45° C. for 1 hour, then made alkaline, aerated and titrated as described for ammonia substantially by the method introduced by Marshall (35) and improved by Van Slyke and Cullen (54).

Uric acid was first isolated by precipitation as silver urate, washed in acidified sodium chloride solution and then determined by the colorimetric method of Benedict (4).

Creatine was converted into creatinine by heating on the water bath with hydrochloric acid as recommended by Folin and the total creatinine was determined by the Folin colorimetric procedure against a standard creatinine solution.

Amino acids were determined first by the formol titration method of Henriques and Sörensen (30) later by the colorimetric method of Folin (19), neither of which seemed altogether satisfactory though results by the two methods agreed fairly well.

Hippuric acid was determined by the method of Kingsbury and Swanson (31) which seemed to give consistent results and good recoveries of added material.

The determination of allantoin was attempted by several different methods none of which was completely successful. The simplest and most successful was that of Larson (34). Even with this, allantoin has proved the most unsatisfactory determination attempted in this work.

Total phosphates were determined by the method of Fiske and Subbarow (16).

Total sulphates, also inorganic sulphates, were determined by the method of Folin (20).

Chlorides were determined by the Arnold modification of the Volhard method (28).

Calcium was determined by the McCruden method (38).

Sugar was determined by the Bugbee and Simond (9) modification of the Shaffer and Hartman method (51).

Two sets of sugar determinations were made: (a) on the untreated samples and (b) on samples treated to remove other reducing substances by successive precipitations with phosphotungstic acid, lead acetate and sulphuric acid.

RESULTS AND DISCUSSION OF URINE ANALYSIS

The proportion of total nitrogen and of urea nitrogen found in the urine of both groups of cows is shown in Table 4 as is also the daily nitrogen excretion in each form. The excretion of total nitrogen averages 9.6 times as great in the high protein group as in the low, while the

TABLE 4.—Daily Excretion of Total Nitrogen and Urea Nitrogen in Urine

Sample No.	Volume Urine	Total Nitrogen	Total Nitrogen	Urea N	Urea N Daily Exc.	Total N as Urea
	(ml.)	(mg./ml.)	(g.)	(mg./ml.)	(g.)	(%)
			Low Protein Ration			
293 1	6,200	3.90	24.18	.28	1.74	7.1
2	5,670	3.46	19.62	.41	2.32	11.8
3	10,000	3.44	27.86	.40	4.00	14.3
4	4,920	4.56	22.44	.56	2.76	12.3
5	8,200	5.32	43.62	.40	3.28	7.5
301 1	12,700	1.52	19.30	.03	.38	1.97
2	8,500	2.34	19.30	.03	.38	17.5
3	7,520	1.85	13.90	.06	.45	3.24
4	12,920	1.20	15.50	.24	3.10	20.0
5	8,240	3.68	30.32	.34	2.80	9.23
Average	8,487	3.13	23.66	.28	2.43	10.49
			High Protein Ration			
292 1	12,800	20.32	260.1	16.88	216.1	83.1
2	13,870	19.40	269.1	18.20	252.4	93.8
3	13,580	18.70	254.0	16.20	189.2	74.5
4	11,680	18.90	220.8	15.00	175.2	79.3
5	13,440	18.64	250.5	14.60	196.2	78.3
329 1	12,800	16.20	207.4	12.64	161.8	78.0
2	8,980	23.37	209.9	17.60	158.0	75.2
3	9,230	21.70	195.7	15.80	164.3	83.9
4	7,720	23.30	179.9	20.00	154.4	85.8
5	9,400	16.26	152.9	12.80	120.3	78.7
332 1	13,000	16.86	219.2	10.56	137.3	62.2
2	12,410	19.36	240.3	16.10	199.8	83.1
3	10,990	20.00	219.8	17.40	191.2	87.0
4	14,800	24.60	364.1	20.80	307.8	84.3
5	17,120	10.80	184.9	8.40	143.8	77.7
Average	12,121	19.22	228.6	15.53	184.5	80.7

excretion of urea nitrogen is 76 times as great. These ratios are by no means constant, however, varying widely between individuals in each group, also with the same individual at different times of lactation as more or less protein was required for milk production. The average proportion of total nitrogen occurring as urea was 10.5 percent for the low protein, and 80.7 percent for the high protein group of cows. The proportion of total nitrogen occurring as urea in a group of 16 cows which were fed on a ration of alfalfa hay corn silage and a grain mixture of about 14 percent protein content, ranged from 59 to 71 percent with an average of 65.9 percent.

The observed proportion and average daily excretion of the nitrogenous constituents other than urea are shown in Table 5. The value for ammonia though small is more than eight times as great in the high protein group as in the other. It was soon learned that if ammonia determinations were attempted on samples stored for only a few hours the results were strikingly higher particularly for the high protein group, probably because of a spontaneous breakdown of urea to ammonia. This may be the explanation of much higher ammonia values found by some other investigators who have worked on stored samples. The

TABLE 5.—Nitrogenous Constituents Other Than Urea in the Urine

Observed Constituent	No. Obs.	Values			Daily Excretion
		Highest	Lowest	Average	
		(milligrams in 100 ml.)			(g.)
		Low Protein Feeding			
Ammonia N	16	3.3	0.0	1.1	.078
Amino Acid N	8	64.0	20.0	40.0	3.400
Uric Acid N	12	18.3	5.0	10.6	.83
Total Creatinine N	10	107.0	27.0	61.9	4.83
Allantoin N	13	630.0	117.0	454.1	15.3
Hippuric Acid N	9	----	----	113.6	9.65
		High Protein Feeding			
Ammonia N	21	9.6	1.4	5.5	.646
Amino Acid N	12	51.0	9.0	30.0	3.50
Uric Acid N	18	12.3	4.2	6.7	.76
Total Creatinine N	12	63.0	26.0	44.2	5.34
Allantoin N	15	550.0	140.0	301.0	14.6
Hippuric Acid N	10	----	----	80.3	9.73

writer has shown in another connection (46) that the ammonia content of cows' urine may be increased sometimes as much as 100 fold as a means of neutrality regulation.

The remaining nitrogenous constituents are not markedly different between the two groups and the amounts found are well within the range of normal values found by others. The somewhat higher value for uric acid nitrogen for the low protein group seems to be in line with a similar situation found in the blood and milk of these cows. The group of substances listed in this table accounts for most of the total nitrogen in the urine from the low protein cows.

Table 6 has been introduced in an effort to show to what extent the analysis of the various nitrogenous constituents of the urine account for the total nitrogen as determined separately. Totaled without the allantoin, a determination with which we are not satisfied, approximately 90 percent of the total nitrogen is accounted for in each group. When allantoin is included in the totals there is a surplus of about 52 percent in the low protein group and still a deficiency of about 4 percent in the high protein group. Substantially higher results for urea obtained later on a few samples by a modification of the Xanthydrol precipitation method of Fosse (24) lead to the suspicion that perhaps the enzymatic cleavage of urea may not have been complete, especially in the higher concentrations.

TABLE 6.—Distribution of Nitrogen in Urine, Daily Excretion in Urine of Experimental Cows

	Low Protein Feeding (1:13)*	High Protein Feeding (1:2)*
	(g.)	(g.)
Total Nitrogen (direct determination)	23.66	228.57
Urea Nitrogen	2.43	184.52
Creatine and Creatinine Nitrogen	4.83	5.34
Uric Acid Nitrogen	.83	.76
Amino Acid Nitrogen	3.40	3.50
Ammonia Nitrogen	.09	.64
Hippuric Acid Nitrogen	9.67	9.32
Allantoin Nitrogen	14.80	14.50
Total, without Allantoin	21.25	204.08
Total, including Allantoin	36.05	218.58

*Nutritive ratio of the total ration fed.

Salkowski (49) is said to have been the first to show the presence of allantoin in bovine urine. This determination seems to have been avoided by most other investigators probably because of the difficulties involved in determining. Some of the methods which have been used for allantoin determination depend on converting it into urea. It seems quite possible that similar transformations among these closely related substances may occur inadvertently during the course of analysis or of sample storage thus leading to inaccurate results.

The proportion and daily elimination of the various non-nitrogenous constituents are shown in Table 7. The sugars reported as dextrose both before (a) and after preliminary removal of other reducing materials (b) are seen to show identical figures for concentration in both groups but this leads to about 45 percent greater elinimation in the high protein group because of the greater volume of urine. Calcium and phosphorus are present in only small amounts in the urine of either

TABLE 7.—Non-nitrogenous Constituents in the Urine

Observed Constituent	No. Dets.	Highest	Lowest	Average	24 hour Excretion
		(Milligrams in 100 ml.)			(g.)
	Low Protein Feeding				
Sugars as Dextrose (A)	7	420.0	136.0	280.0	23.24
Sugars as Dextrose (B)	7	142.0	----	75.8	6.3
Calcium	6	35.3	3.2	17.5	1.6
Total Phosphate	6	1.6	----	0.5	.04
Sulphate as H_2SO_4 Total	7	76.0	38.0	53.0	13.7
Sulphate as H_2SO_4 Inorganic	7	62.0	5.0	29.8	2.54
Chlorine	10	1137.0	173.0	426.0	36.2
CO_2 as Bicarbonate, (ml.)	12	351.0	49.0	157.0	12,000
	High Protein Feeding				
Sugar as Dextrose (A)	9	374.0	116.0	280.0	33.9
Sugar as Dextrose (B)	9	190.0	42.0	75.8	9.2
Calcium	9	12.7	1.6	4.9	0.59
Total Phosphate	9	18.9	----	5.5	0.67
Sulphate as H_2SO_4 Total	12	177.0S	95.0S	130.0	46.2
Sulphate as H_2SO_4 Inorganic	12	105.0S	51.0S	78.4	26.0
Chlorine	10	180.0	60.0	143.3	17.4
CO_2 as Bicarbonate, (ml.)	16	187.0	54.0	108.0	13,089

group in spite of fairly large amounts in the ration. A like condition has been noted by other investigators showing that the urine is not an important path of excretion of these materials. Both concentration and elimination of sulphur are much greater for the high protein group, roughly three-fold, probably due to the sulphur supplied by the great excess of proteins in the ration. Just the opposite condition prevails with respect to chlorine.

An hypothesis introduced by Bunge (10) might lead to the assumption that this had been caused by a higher potassium content of the low protein ration. Checking both rations for potassium content from analyses found in the Morrison tables shows 0.32 pound and 0.40 pound per day, respectively, for the low and high protein cows, thus refuting that explanation. A more probable explanation is that the additional chlorine serves as a means of neutrality regulation compensating for the acidity due to sulphur derived from the higher protein content of the other ration. The elimination of CO_2 as bicarbonates, another means of neutrality regulation, is quite similar for both groups.

COMPOSITION OF THE SALIVA: Procedures and Results

In view of the remarkable results observed for the concentration of urea in the blood, milk and particularly the urine of the experimental cows, it was decided to extend our investigation to include the total nitrogen and urea concentration in the saliva of cows in these groups.

Several articles are to be found in the literature showing a rather close parallel between the level of urea to be found in the blood and the saliva. The use of saliva instead of blood for ascertaining the urea level when blood is difficult to procure is suggested in some of these articles. Miyazaki (40) also observed 2-8 fold increases in both blood and salivary urea when high protein foods or urea were given in large amounts. The values soon returned to normal with return to normal diet.

Most of these references deal with humans or with laboratory animals. However, since the completion of these experiments values for protein and non-protein nitrogen content of saliva obtained from cattle have been published (39). Also, recently, McDonald (39) showed that the presence of NH_3 and urea nitrogen in the saliva may provide an important source of non-protein nitrogen for rumen microorganisms.

The saliva samples examined in this experiment were obtained by placing a bit in the cow's mouth. Chewing of the unaccustomed object in the mouth brought on a secretion of saliva which varied in volume

and consistency with the different animals. The cow was tied so that movement of the head was somewhat restricted. The saliva was caught in a large pan or tray and soon transferred to bottles. It was filtered through cotton cloth and sampled for analysis at once. The methods used were similar to those used for determining the same ingredients in milk (43).

The results of these determinations are shown in Table 8. They show that both the total nitrogen and the urea plus ammonia nitrogen are markedly higher for the animals on high protein feeding. In the case of total nitrogen the average is about 2½ times as great and in case of urea the average is about sixe times as great on the high as on the low level of protein feeding.

Ammonia nitrogen was not determined separately on all these samples, however; two samples of saliva from high protein fed cows analyzed for ammonia content by the writer's method for ammonia in milk (45) showed values of 1.0 and 2.4 mg. percent of ammonia nitrogen. These values were 5 percent and 10 percent respectively, of the

TABLE 8.—Nitrogen Content of Saliva from Cows Fed High and Low Protein Rations

(Milligrams per 100 ml.)

TOTAL NITROGEN IN SALIVA

No. Dets.	Highest	Lowest	Mean
	Low Protein Feeding		
5	40	27	32
	High Protein Feeding		
5	106	56	77

UREA NITROGEN PLUS AMMONIA NITROGEN IN SALIVA

No. Dets.	Highest	Lowest	Mean
	Low Protein Feeding		
10	6.4	2.3	3.6
	High Protein Feeding		
9	30.0	17.0	22.0

values for urea plus ammonia obtained on the same samples, showing that most of the combined values for urea and ammonia as shown in Table 8 were due to urea. One sample from a low protein fed cow showed values of 0.4 and 2.3 mg. percent, respectively for nitrogen as ammonia, and as urea plus ammonia.

It is probable also that some of the ammonia found in these samples was broken down from urea during the distillation since the writer has shown (45) that this occurs to a certain extent in distilling a solution of pure urea under like conditions.

SUMMARY

The nitrogenous constituents of blood, urine and saliva were studied in two groups of cows. One group, the low protein, was fed on a ration of nutritive ratio of 1:13 and the other group, the high protein, was fed on a ration of nutritive ratio 1:2.

The total non-protein nitrogen for the high group was more than twice as great as the low protein. Urea nitrogen was found to be eleven times greater for the high group as the low protein group whereas uric acid averaged slightly higher for the low protein group. Small differences were noted in other constituents.

The total excretion of nitrogen averaged 9.6 times as great in the high protein group as in the low, while the excretion of urea nitrogen was seventy-six times as great. Urine ammonia, although a small proportion of the total urine nitrogen, was 8 times higher in the high protein group than in the low protein. Other nitrogen constituents did not differ markedly between groups.

Analysis of samples of saliva showed that both the total nitrogen and urea plus ammonia nitrogen were markedly higher for animals on high protein feeding. The urea nitrogen being about six times greater in the high protein group showing that a considerable amount of blood urea was recycled into the rumen when cows were fed very high levels of protein. However, when the urine data was considered, a much greater proportion of the blood urea was excreted through the kidneys.

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